MDM2 binding site, D21E and K24N. The aspartic acid and lysine residues at positions 21 and 24 respectively, stabilize the helix of human p53 when bound to MDM2. D21E and K24N point mutants were created in human p53TAD using site directed mutagenesis. We are currently investigating how these mutants affect the interaction with MDM2.

1. Cheng Y, LeGall T, Oldfield CJ, Mueller JP, Van YY, Romero P, et al. Rational drug design via intrinsically disordered protein. Trends Biotechnol. 2006;24(10):435-42.

311-Pos Board B111

Conformational Properties of Polyglutamine Sequences Under Denaturing Conditions

Khurshid A. Khan, Bruce E. Bowler.

A number of diseases are caused by polyglutamine sequences in proteins such as Huntington's disease, spinocerebellar ataxia and many more. A better insight into the behavior of polyglutamine sequences is essential for understanding their role in diseases. Therefore, it is worth studying the behavior of polyglutamine sequences in one of the early steps of protein folding, i.e. loop formation. To this end, we are looking into the thermodynamics and kinetics of loop formation of polyglutamine sequences using engineered yeast iso-1-cytochrome *c*, which forms a loop between His(-2) and the heme in the denatured state. The His(-2) is part of a five amino acid N-terminal extension which is not found in eukaryotic cytochrome *c* and readily accepts homopolymeric inserts. All the inserts are between His(-2) and Ala(-1). Five variants were made in such a way that each variant contains 1 to 5 sets of the sequence QQQQQK. The lysine is present in order to maintain solubility. Stability of these variants was measured by CD spectroscopy and is compared with the previously reported polyalanine inserts [Tzul, F. O and Bowler, B. E. *Proc. Natl. Acad. Sci. USA, 107*, 11364-9 (2010)]. pH titration experiments were performed in 3 M and 6 M guanidine-HCl to measure equilibrium His-heme loop formation with all variants. The scaling exponents for loop formation are compared with those of polyalanine inserts in 3 M and 6 M guanidine-HCl, respectively. Kinetics of loop formation and loop breakage for polyglutamine variants were performed by pH jump methods in both 3 M and 6 M GdnHCl. The kinetics are compared with the previously reported data for polyalanine sequences and with data for His-heme loop formation in the denatured state of *Rhodopseudomonas palustris* cytochrome *c*'.

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Probing the Role of Disordered N- and C-Terminal Regions During α-Synuclein Fibril Assembly

Thai L. Yap, Candace Marie Pfefferkorn, Jennifer Chen Lee.

α-Synuclein (α-syn) is a 140-residue, cytoplasmic, and membrane-associated protein localized predominantly in the presynaptic terminals of neuronal cells. Upon aggregation, α-syn undergoes a large conformational change from an intrinsically disordered monomer to parallel, in-register and highly-ordered β-sheet containing fibrils or amyloids. Importantly, the amyloid form of α-syn is the major proteinacious constituent in Lewy bodies, pathological hallmarks of Parkinson's disease. To date, the α-syn fibril core has been indentified to be comprised of residues 30-100; however, the molecular details pertaining to the disordered N-and C-terminal regions in fibril formation remains ill-defined. Here, we report the use of single-Cys mutants derivatized with an environment sensitive dansyl flurophore to provide residues-specific observations during the assembly events of monomeric α-syn to fibrils. Interestingly, spectroscopic signatures of fluorophores attached to residues in the disordered regions are more sensitive to the early aggregation stages compared to that of residues at the fibril core region. These residue-specific data support the proposals that the disordered N-and C-terminal regions participate in interchain contacts prior to fibril assembly and they may play a key role in the interactions between protofilaments in forming the mature fibrils.

313-Pos Board B113

WWW.DISPROT.ORG: The Database of Disordered Proteins

Caron Morales.

The Database of Protein Disorder (DisProt) links structure and function information for intrinsically disordered proteins. Intrinsically disordered proteins (IDPs) do not form a fixed three-dimensional (3-D) structure under physiological conditions, either in their entireties or in segments or regions. We define an IDP as a protein that contains at least one experimentally-determined disordered region. Although lacking fixed structure, intrinsically disordered proteins and regions carry out important biological functions, being typically involved in regulation, signaling, and control. Such functions can involve high-specificity-low-affinity interactions, the multiple binding of one protein to many partners, and the multiple binding of many proteins to one partner. These three features are all enabled and enhanced by protein intrinsic disorder. One of the major hindrances in the study of IDPs has been the lack of organized information. DisProt was developed to facilitate IDP research by collecting and organizing knowledge regarding the experimental characterization and the functional associations of IDPs. In addition to being a unique source of biological information, DisProt opens doors for a plethora of bioinformatics studies. DisProt is openly available at http://www.disprot.org.

314-Pos Board B114

Synergistic Folding and Binding of Two Intrinsically Disordered Proteins

Debabani Ganguly, Jianhan Chen.

The nuclear coactivator-binding domain (NCBD) of CREB binding protein is an intrinsically disordered protein (IDP), which exists as molten globule like structures in the unbound state. As one of the most folded IDPs, NCBD folds synergistically with another IDP, activator for thyroid hormone and retinoid receptor (ACTR) of the p160 steroid receptor. A topology-based Go-like coarse-grained protein model has been used to investigate the mechanism of the NCBD:ACTR interaction. The simulation results support a largely cooperative mechanism for the folding of the two IDPs. Specifically, while the binding induced folding follows multiple pathways, the α2 helix of ACTR most frequently initiates the binding by interacting with a preformed structural motif of NCBD, where the α2 and α3 helices of NCBD are mostly folded and correctly packed. This initial binding is followed by the binding and folding of rest of the helices of both IDPs in a highly cooperative fashion. This is consistent with the importance of the disordered leucine-rich motifs in specificity of NCBD:ACTR established by previous biochemical and biophysical data, and further supported by unpublished mass spectroscopy data from David Weis's lab. Compared to ACTR, folding of NCBD appear to mostly involve assembly of pre-folded of α1 and α2 helices, and only α3 folding appears to be initiated by ACTR binding.

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Understanding Disordered Protein Sub-Groupings by Four-Quadrant-Plot Derived from Vsl2 and Charge-Hydropathy Plot Disorder Prediction

Fei Huang.

Intrinsically disordered proteins do not fold under normal physiology conditions. Proteins can be fully structured, full disordered, or partially disordered. Because of the non-folding and flexibility exhibited by disordered proteins, they carry out quite distinguishable functions from fully structured proteins. However, in reality, it is difficult to place a binary conclusion about a protein as disordered or ordered, because the protein can have both structured and disordered domains. Therefore, we employed the differences of attributes in disordered protein predictors to further categorize disordered proteins into three sub-groups. Each group is shown to contain proteins with their own structural and functional features. According to our grouping method, one sub-group of disordered proteins is specialized for nucleotide binding. Another sub-group, hypothesized as collapsed disordered proteins, is highly involved in cell signaling regulations. Meanwhile, the extended disordered group is shown to be important for cell division. Interestingly, the second group is only abundant in multi-cellular organisms, especially in vertebrates. Their regulatory functions play important roles in cell differentiation and organism development.

316-Pos Board B116

Prediction of Binding Sites on Intrinsically Disordered Proteins

Russell C. Goodman, Theresa L. Beaty.

Many of the methods for analyzing intrinsically disordered proteins (IDPs) to date have been limited to sequence analysis that attempts to predict intrinsically unstructured regions of proteins. However, our research is based on developing algorithms for predicting ligand binding sites and the associated secondary structure of these binding sites in IDPs. Our algorithms are founded on parameters determined through a statistical method. This method used bound IDP structures in the Protein Data Bank to calculate the frequency of amino acids occurring at the binding regions of eight nucleic acid binding IDPs. Our primitive sequence composition algorithm for predicting binding sites, SeqCom, predicts, on average, 83.2 percent of the binding sites with 47.0 percent of the binding sites predicted representative of the native binding regions. To improve binding site prediction, we developed IUPattern. IUPattern works on the same principles as SeqCom, but it uses additional constraints to better decipher between native and non-native binding sites. IUPattern predicts, on average, 90.2 percent of the binding sites with 55.0 percent of the binding sites predicted correctly correlating the native structure. Currently, we are finalizing a dynamic programming algorithm which predicts binding sites and secondary structure on nucleic acid binding IDPs. This algorithm incorporates an annotated sequence to show our confidence in the predictions.